

THE DEVELOPMENT OF HEMATOLOGICAL PROFILE IN EXPERIMENTAL GROUPS OF BROILER CHICKENS GIVEN OVERDOSES OF OXYTETRACYCLINE

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Abstract

In the intensive rearing systems for broiler chickens, a common practice is the overdosage of antibiotics in order to cure or prevent diseases or as growth promoters. Despite all the advances in medicine and technology, the residual antibiotics in poultry meat are still an important problem, generating controversy between the farmers, who support the benefits of antibiotics and the final consumers, who bring up the health risk regarding the food safety of these substances. This research aims on evaluating the effects of overdosing an oxytetracycline based product, on the hematological parameters in chickens, for an early detection of such risks. The hematological effects of overdosing an oxytetracycline based product (Galiprotect) were evaluated on five experimental groups, each consisting of 11 broiler chickens, receiving therapeutic, double and higher doses (8 times and 16 times the therapeutic dose), for three consecutive days; also a control group was kept and fed in the exact same conditions as the experimental groups. After the treatments and at the end of the experimental period, the main hematological parameters have been determined and the recorded data was statistically analyzed and interpreted. The individual values of the hematological parameters presented important variations, some outside the physiological ranges. Their evolution indicated that the overdose of the oxytetracycline based product affected mainly the leukocyte population, inducing leukocytosis ($p < 0.0001$) also associated with heterophillia ($p < 0.0001$) and lymphopenia, respectively, attributed to the AD3E vitamin complex from the drug formula. The statistical analysis also indicated decreases of the erythrocyte parameters, a statistically significance being recorded for the hemoglobin (0.0027^D) and the MCHC values ($0,0007^D$). The therapeutic use of the oxytetracycline based products in poultry determines hematologic changes, expressed by leukocytosis associated with heterophillia and decreases of the erythrocyte mass parameters, based on which the overdosage can be suspected.

Key words: oxytetracycline, multiple doses, broiler chicken, hematology.

INTRODUCTION

The optimum dose is given by the quantity of active substance necessary to attain the therapeutic effect, as a response of the organism to the administration of the drug through a certain route.

The optimum dose also represents the quantity of active substance which determines plasmatic concentrations and distribution rates that are specific for certain active substances. The therapeutic index (safety limit) and the elimination rate ($T_{1/2}$) of the active substances from a drug formula represent the factors that limit the dose and

the duration of action (CRISTEA, 2009; OGNEAN et al., 2010).

Maintaining the plasmatic concentrations to therapeutic levels requires the use of successive doses, at certain time periods; this dosage regime serves to maintain the minimum inhibitory concentration (MIC). Thus a stable concentration can be distinguished, that represents the time in which the concentration remains at a certain level (plateau phase) after the administration of the medical product (BAGGOT, 1977; OGNEAN et al., 2010).

The therapeutic plasmatic concentration depends on the size of the dose, the interval between two administrations, the route of

administration, the systemic distribution, the rate and degree of absorption, the rate of binding to plasmatic proteins and the rate of elimination (BAGGOT, 1977). The following study is intended to comparatively evaluate the effects of the therapeutic and multiple dosage of an oxytetracycline based product, on the hematological parameters in meat chickens, for an early detection of the risks generated by an overdose.

MATERIALS AND METHODS

The set-up of the experiment was based on an initial analysis of the general conduct procedures for applying medical treatments on a large scale, by means of medicated feed or in drinking water. In these types of procedures there is always a risk of occurring potential dosing errors, with severe consequences when overdosing. In our experiment the accidental overdosing was excluded, due to the fact that the product used was administered as tablets (Galiprotect tablets-Romvac product), which was administered by oral route.

Five groups of broiler chickens were taken into the study (n=15) from the Ross line 308, 9-14 days of age, with a weight of over 250 grams, coming from 2 commercial farms; the experimental variables were: therapeutic dose, double dose, eight times higher and 16 times higher than the normal dose. The chickens from the experiment were organized in the following groups: I- chickens treated two consecutive days with the recommended dose (1/4 tablet/day/animal); II- chickens treated for two consecutive days with a double dose (1/2 tablet/day/animal); III- chickens treated for two consecutive days with eight times the therapeutically dose (2 tablets/day/animal); IV- chickens treated for two days with 16 times the therapeutically dose (4 tablets/day/animal); V- control group, kept and fed in identical conditions with the treated groups.

Blood samples were collected post-treatment from the birds in the experimental groups and at the end of the experiment from the control, using EDTA as anticoagulant; the blood samples were collected by puncturing the basilar vein.

The investigated hematological parameters regarded complete cell counts (number of erythrocytes and leukocyte), hematocrit (Ht), hemoglobin (Hb), mean erythrocyte constants (MCV, MCH and MCHC) and differential leukocyte counts (H, E, B, L, M). Hemocytometric method was used to determine the total number of erythrocytes and leucocytes; a spectrophotometer was used to determine the hemoglobin concentration and micro-hematocrit method for PCV. The mean erythrocyte constants were determined using the aforementioned parameters and the differential leukocyte counts were performed on panoptically stained smears.

The recorded data was statistically processed and interpreted using specialized applications (GraphPad InStat, OriginPro), using the Tuckey statistical test and Dunn test for non-parametric distributions.

RESULTS AND DISCUSSIONS

From the recorded data it appears that there is very little information available regarding the influence of the medical substances on the hematological parameters in meat chickens raised in intensive farms, which justifies our investigations in this study and their continuation in the following research. For the chickens in the control group, the values recorded for the hematological parameters fall within physiological ranges, confirmed by most references, which justifies the use of the results.

Among the specific characteristic for the erythrocyte parameters evolution (table 1) is worth to notice the dynamics, without any statistical significance ($p=0.9500$), of the mean hematocrit values: $39.59 \pm 3.69\%$ for the group treated with the therapeutic dose; $39.45 \pm 2.94\%$ for the group treated with a double dose; $39.55 \pm 4.90\%$ for the group with a dose 8 times higher and $39.69 \pm 8.36\%$ for the group treated with a dose 16 times higher than the therapeutic dosage.

The hemoglobin values showed fluctuations of the mean results, between a minimum of 6.40 ± 0.84 g/dl (in the group treated with the 16 times higher dose) and a maximum of 8.00 ± 0.65 g/dl (in the group treated with the

single dose), with a probability index „p” (0.0027), corresponding to statistically significant differences.

The total number of erythrocytes showed no statistically significant changes and was characterized by mean values of 2.44 ± 0.36 T/l for the group treated with the therapeutic dose; 2.72 ± 1.01 T/l for the group treated with a double dose and 2.72 ± 1.01 T/l for the group exposed to a dosage eight times higher the therapeutic dose, respectively 2.25 ± 0.47 T/l

for the group treated with a dosage 16 times higher.

The same trend was also recorded for the mean erythrocyte values, which showed tight mean results, with no statistical significance, except for the MCHC values, which showed significant statistical differences when comparing the group treated with the 16 times higher dose and the control group (table 1).

Table 1. Mean values of the erythrocyte parameters and probability index

Group	Parameter	Dose				Control	„p” index
		Therapeutic	Double	Multiple (8x)	Multiple (16x)		
		Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	
E	(T/l)	2,44±0,36	2,63±0,19	2,72±1,01	2,25±0,47	2,46±0,45	0,2797
Hb	(g/dl)	7,90±0,62	8,00±0,65	7,10±1,12	6,40±0,84	9,04±3,30	0,0027 ^D
Ht	(%)	39,59±3,69	39,45±2,94	39,55±4,90	39,69±8,36	39,56±7,09	0,9500
MCV	(fl)	164,96±25,37	150,77±15,78	172,67±64,90	177,00±19,38	167,19±40,77	0,4963
MCH	(pg)	33,08±5,71	30,53±2,92	29,50±8,29	29,65±7,59	37,37±13,62	0,2013 ^D
MCHC	(g/dl)	20,18±2,87	20,31±1,47	18,17±3,50	16,69±3,56	23,10±6,87	0,0007^D

^D= Value calculated through Dunn test for non-parametric distributions

The analysis of the leukocyte profile revealed individual fluctuations, of more or less importance, with the means and standard deviations showed in table 2. Thus, for the total number of leukocytes, the mean values displayed variations in the range of 21.53 ± 2.68 and 24.95 ± 9.56 G/l, the minimum being recorded for the group treated with the therapeutic dose and the maximum for the group treated with a dosage eight times higher than the therapeutic dose.

The evolution of the heterophils subpopulation indicated mean values fluctuating between a minimum of $41.27 \pm 5.14\%$ (recorded for the group treated with the therapeutic dose) and a maximum of $61.55 \pm 5.91\%$ (in the group treated with a dose eight times higher). The observed differences for this parameter proved to be of high statistical significance ($p < 0.0001$). The eosinophil values showed mean values ranging between $1.27 \pm 0.79\%$ (in the case of the group treated with a therapeutic dosage) and $4.00 \pm 2.24\%$ (recorded for the group

treated with a double dosage). The value ranges of the lymphocyte population varied between $21.27 \pm 5.78\%$ and $42.45 \pm 4.48\%$, the minimum value being recorded in the group treated with the eight times higher dose than the therapeutic dosage and the maximum value being recorded in the case of the group treated with the therapeutic dose. The reported differences were statistically significant ($p < 0.0001$). Significant increases were also recorded for the monocyte populations, with mean results ranging between 13.27 ± 4.52 and 17.00 ± 6.47 , the recorded differences being statistically significant ($p = 0.0053$).

Knowing that heparin is not suited for the determination of fibrinogen and can lead to errors when it comes to counting the total number of leukocytes (CLARK et al., 2009), EDTA was used as an anticoagulant; also, the use of Natt liquid allowed a good identification of the leukocytes.

Table 2. Mean values of the leukocyte parameters and probability index

Group	Parameter	Dose				Control	„p” index
		Therapeutic	Double	Multiple (8x)	Multiple (16x)		
		Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	Mean ± st. dev.	
Leuc.	(G/l)	21,53±2,68	24,37±2,93	24,95±9,56	22,95±4,91	18,28±6,10	<0,0001
H	(%)	41,27±5,14	40,45±2,70	54,55±9,84	61,55±5,91	48,85±9,40	<0,0001
E	(%)	1,27±0,79	4,00±2,24	4,45±3,14	3,64±2,66	2,40±1,97	0,0033 ^D
B	(%)	1,73±1,10	1,18±0,98	0,00±0,00	0,00±0,00	0,39±0,80	<0,0001 ^D
L	(%)	42,45±4,48	37,36±6,74	25,64±12,46	21,27±5,78	36,77±8,54	<0,0001
M	(%)	13,27±4,52	17,00±6,47	15,36±7,24	13,55±4,89	11,59±4,86	0,0053

^D= Value calculated through Dunn test for non-parametric distributions

The evolution of the hematological parameters revealed small changes, of no statistical significance. This fact indicates that the therapeutic dosage of the two products does not have a relevant influence on the hematological and hematopoietic profile. On the other hand, the dynamics of the hematological changes in the group treated with a higher dose revealed important variations, with statistical significance in the case of the hematological parameters. These changes did not prove that the accidental overdose with antibiotics from the tetracycline group, as a cause of technological malfunctions or of the automatic therapy systems, are well tolerated, without inducing iatrogenic symptoms, with acute evolution and immediate clinical consequences.

As it shows from our results, the overdose with oxytetracycline hydrochloride based products, affects mainly the leukocyte profile, leading to leukocytosis and heterophilia. Similar results were reported by other researchers (Al-Mayah et al., 2005; Turcu et al., 2011), considering that this tendency of lymphopenia can be caused by the vitamins A, D₃ and E, also present in this drug formulation.

Among the significant findings observed in this study, it is worth mentioning the gelification of the blood plasma in several samples. This process, represented by the total or partial transformation of the plasma in a gelatinous mass, without figurative elements, similar to a blood clot, can affect to 25% of all the aviary blood samples, totally or partially compromising the plasma (HARR, 2006). In following research, the plasma gelification affected 3 samples from 15, thus

reducing the number of plasma samples obtained from the experimental groups. Noteworthy are also the results obtained regarding the ratio of different cell populations. According to our data, for the healthy chickens, also relevant were the erythrocytes/leukocytes ratios (1:155), along with those for the leukocyte population (L/H-1:1.47; M/H -1:5.7; M/L -1: 4.25).

It is advisable that the health surveillance of the broiler chickens requires screening type assessments of the hematological and biochemical profile, these types of tests being useful also in the case of suspected accidental drug overdose.

CONCLUSIONS

The changes of the hematological profile reported in the birds treated with multiple doses revealed that the overdose with tetracycline is well tolerated, without producing side effects of major concern.

In order to explain the secondary lymphopenia present after the overdose with the tested products we must highlight to the content in vitamins A, D₃ and E.

Some of the hematological alterations, like the ones expressed by the anemic syndrome in the case of oxytetracycline, can be the basis of the overdose with antibiotics in birds.

Some of the blood samples (20%) were affected by gelification (coagulation), in a short period of time after the blood was collected, a process that determined the partial or total compromise of the samples.

The blood reports showed ratios characteristic for the species and age of the birds, between

the L/H (1:1.47), M/H (1:5.7) and M/L (1:4.25).

ACKNOWLEDGMENT

The authors are thankful to SC Romvac Company SA for the financially supported by this study.

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